3327-Pos Board B55
Moonlighting Proteins
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Moonlighting proteins comprise a class of multifunctional proteins in which a single polypeptide chain has multiple biochemical functions that are not due to gene fusion events. Examples include cytosolic enzymes that are also transcription factors, crystallins, chaperones, extracellular growth factors, or cell surface adhesins. The variety of known moonlighting proteins, the multiple ways in which one protein can have multiple functions, the potential benefits to the organism of combining two functions in one protein, and the methods proposed for a protein to evolve a second function suggest that moonlighting proteins might be common. The ability of a protein to moonlight in different multi-protein complexes or pathways can complicate the prediction of protein function from sequence or structure and the annotation of sequence databases. To date, most moonlighting functions have been found by serendipity. There is currently no straightforward method to identify which proteins moonlight, or for determining if a protein of interest is a moonlighting protein. In addition, sequence homologues of moonlighting proteins often do not perform both functions. We are using biochemical methods and X-ray crystallography to study individual moonlighting proteins. We are also organizing information about the sequences, structures, functions and functional sites of the over 200 known moonlighting proteins into the web-based MoonProt database.

Dynamics of Ligand Binding

3328-Pos Board B56
Thiol Labeling Reveals Presence of Cryptic Binding Sites in β-Lactamase
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The field of drug design has for many years sought to design ligands for known binding pockets in proteins. Cryptic binding sites, which are unexpected openings that are invisible to normal experimental methods but can modulate activity through communication with the active site, have the ability to greatly expand the number of proteins whose behavior and activity can be easily modulated by increasing the number of druggable sites. Recent work has offered the possibility of identifying these sites from molecular dynamics simulations of proteins in the absence of ligands, allowing identification of sites for many ligands to be screened in a single experiment. To experimentally verify these computational models, we have tested β-lactamase for the presence of cryptic binding sites. We have employed a method using mutagenesis and thiol labeling to verify the transient opening of cryptic sites as well as the ability of a large, covalent adduct at these sites to alter activity of the protein. This has led us to conclude that conformations with transiently open pockets exist in equilibrium with the known structure under native conditions, and these pockets may be suitable for future drug design studies.

3329-Pos Board B57
Structural Dynamics Studies of Fatty Acid Binding Protein-4 by Solution NMR Spectroscopy
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Adipocyte fatty acid binding protein-4 (FABP4) is a 132-aa intracellular lipid binding protein involved in the transport of fatty acids between cell membranes and organelles. FABP4 participates in several pathways including lipolysis and lipogenesis, and is involved in lipid and energy metabolism related diseases such as diabetes. Although the x-ray structure of FABP4 has been determined and binding to several of its hydrophobic ligands well characterized, the transitions in the structural dynamics upon ligand binding has yet to be determined. Here, solution NMR experiments will be carried out on 15N and 13C labeled FABP4 to study the structural transitions between its free and bound states. Spin relaxation measurements will also be used to reveal any changes that occur upon binding of FABP4 to its hydrophobic ligands.

3330-Pos Board B58
Insights into the Cyclic Nucleotide Selectivity Mechanism of Cyclic GMP Dependent Protein Kinase II
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Membrane bound type II cGMP dependent protein kinase (PKG II) is a central mediator of cGMP signaling cascade, which regulates circadian rhythmicity, intestinal water secretion, bone growth and renal functions. PKG II contains an N-terminal regulatory (R)-domain, and a C-terminal catalytic (C)-domain. The R-domain contains tandem cyclic nucleotide binding domains (CNB-A and B) each with different affinities for cGMP, the second messenger that regulates kinase activity of PKG II. While it is known that PKG II needs to be highly selective for cGMP over cAMP to function properly, little is known about its cyclic nucleotide selectivity and the selectivity's role in activation. To understand its cyclic nucleotide selectivity and activation mechanism of PKG II, we first identified CNB-B to be highly selective for cGMP and solved its crystal structure with cGMP. The complex structure revealed that PKG II utilizes an arginine and two aspartate residues on the C-terminal helix to recognize the guanine moiety in cGMP. This is completely different from PKG I, where a conserved arginine from the β barrel of CNB-B specifically binds the guanine moiety of cGMP and imparts cyclic nucleotide selectivity. We are currently testing the roles of PKG II specific interactions in cGMP selectivity and activation of PKG II.

3331-Pos Board B59
Effects of Ligand Binding on the Rigidity and Mobility of Proteins: An Experimental and Computational Approach
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Cyclophilin A is an enzyme which plays a role in the folding of proteins. It also binds to and aids the function of the immunosuppressant drug cyclosporin A as well as binding to the HIV-1 capsid protein.
We expand upon a method recently used in our study of HIV-1 protease and use the computational tools FIRST and FRODA to model the flexibility and motion of cyclophilin A in the presence and absence of its principal ligands.
In order to verify the simulation process, we have also conducted hydrogen-deuterium exchange NMR (HDX) experiments, the results of which are also presented.
The computational procedure is far quicker than more in-depth approaches such as molecular dynamics, yet it yields results which are comparable with experimental data, on a timescale of CPU-minutes.
We use the computational tools FIRST and FRODA to model the flexibility and motion of cyclophilin A in order to predict the results of hydrogen-deuterium exchange NMR (HDX) experiments.
The rigidity analysis software FIRST can be used to predict the "folding cores" of proteins identified as slowly exchanging residues in HDX. This prediction is improved using the protein mobility software FRODA.
We are using these methods to investigate the effect of ligand binding on cyclophilin A computationally and experimentally.

3332-Pos Board B60
Bridging Simulations and Calorimetry: Computational Studies of Binding Thermodynamics and Entropy-Enthalpy Transduction
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Molecular simulations are now fast enough that we can begin to make connections to the thermodynamic data provided by isothermal titration calorimetry. Here, I will describe the thermodynamic analysis of a millisecond-duration protein simulation. A central result is that local perturbations of a protein, due, for example, to binding of small molecules, may easily induce global conformational shifts with large associated shifts in entropy and enthalpy. Thus, the global conformational shifts can effectively transduce local binding thermodynamics into quite different overall apparent thermodynamics. This phenomenon, which may be termed entropy-enthalpy transduction, likely occurs in many systems, and could make measured entropies and enthalpies of binding unreliable indicators of binding forces. Entropy-enthalpy transduction may also help explain the high experimental variance of measured enthalpies and entropies relative to free energies, and may underly many cases of entropy-enthalpy compensation.

3333-Pos Board B61
Differential Responses of Msh2/6 and Damaged DNA Probed by Molecular Dynamics
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Over the past decade, there has been a growing interest in studying the binding of DNA to the MutSalpha protein complex. This heterodimeric protein complex, the Msh2/Msh6 complex in humans, is the initial complex that can

bind mismatched DNA and other DNA defects that may occur during replication. This complex has also been shown to bind at least some types of damaged DNA, such damages due to chemotherapeutics. We use molecular dynamics simulations to model the changes in the protein/DNA and protein/protein interfaces that occur during such binding events.

3334-Pos Board B62

Atomic Resolution Mechanism of Cd44-Hyaluronan Protein-Carbohydrate Binding

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All-atom explicit-solvent molecular dynamics simulations with advanced sampling methods were applied to completely determine, at atomic resolution, the mechanism of binding of the protein CD44 with the glycosaminoglycan carbohydrate hyaluronan. The results include descriptions of: formation of the protein-carbohydrate complex from separate protein and carbohydrate; conformational switching at the binding site to enhance binding affinity; additional binding affinity enhancement through an order-to-disorder transition in the protein; and inhibition of binding by glycosylation of CD44. These computational results are discussed in the context of and illuminate existing experimental data.

3335-Pos Board B63

A Molecular Dynamics Investigation of the Bacterial Cis-Prenyl Transferases: Perspectives on Conformational Flexibility and Chain Elongation Mechanisms

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E,Z-farnesyl diphosphate synthase (E,Z-FPPS; Rv1068) and cis-decaprenyl diphosphate synthase (DPPS; Rv2361c) are promising drug targets in Mycobacterium tuberculosis, the causative agent of tuberculosis, due to their essential roles in the early steps of isoprenoid synthesis during the bacterial cell wall biosynthesis. In this work, we performed a series of molecular dynamics (MD) simulations on E,Z-FPP and DPPS both free and in complex with various ligands in order to investigate their dynamic behaviors. Binding pocket volume calculations obtained from the MD trajectories identified several transitions between open and closed states, indicating considerable structural plasticity of the active sites of these proteins, with the largest flexibility found with DPPS. MD simulations on the mutated E,Z-FPPS and DPPS suggested a possible chain elongation mechanisms for the two enzymes which work in series to synthesize the long decaprenyl diphosphate. To incorporate the flexibility of DPPS into our drug discovery protocol, we carried out virtual screening of a library of 39 known DPPS inhibitors with different receptor conformers obtained from the MD simulations, finding better predictive performance with several rarely sampled MD structures than with static, X-ray crystallographic structures. Our results highlight the importance of accounting for protein flexibility in computer-aided drug design targeting E,Z-FPPS and DPPS.

3336-Pos Board B64

Study of the Dynamic and Thermodynamic Calcium Induced Transition in the Downstream Regulatory Element Antagonist Modulator (DREAM) using Photothermal Beam Deflection (PBD)

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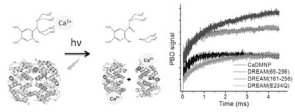
Here we show the implementation of photothermal beam deflection (PBD) as a useful label free technique for the measurement of millisecond and submillisecond kinetics. Moreover, by taking advantage of the photosensitive calcium chelating compound DM-nitrophen, we have been able to photo trigger the release of calcium and consequent binding to four DREAM constructs. The PBD traces for photolysis of Ca^{2+}DMNP show a fast 300 μs decay, whereas upon addition of DREAM(65-256) an additional slow phase with a decay of 1 ms can be resolved. Moreover, a construct lacking the first 161 amino acids shows a slower 5 ms decay. The differences in kinetics can be correlated with the oligomerization transition associated with each construct, where DREAM(65-256) undergoes a tetramer to dimer and DREAM(161-256) a monomer to dimer transition upon calcium binding. Furthermore, the slow transition is measured to be exothermic with a concomitant volume expansion. Together, these results establish the implementation of PBD for dynamic and thermodynamic study of calcium induced transitions in DREAM protein.



3337-Pos Board B65

Solid-State NMR Characterization of S31N M2 Transmembrane Domain Bound to Novel Adamantanes with Persistent *In Vitro* Efficacy

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The M2 proton channel from Influenza A virus is essential for the viral lifecycle and is an important drug target. Amino acid mutations in the residues lining the pore of the channel have abrogated clinical efficacy of the previously FDA-approved antiviral agents, amantadine and rimantadine. More than 95% of the circulating strains isolated from human clinical cases bear S31N mutation and a subsequent resistance to the licensed small molecule inhibitors. Efforts in rational drug design targeting S31N M2 channel have been impeded by the limited number of experimental techniques with capabilities for structural characterization of the protein-ligand interaction in native-like membrane mimetic environments. Solution and Solid State Nuclear Magnetic Resonance (NMR) investigations of the recently introduced adamantane analogues suggest multiple orientations of the inhibitor molecules bound in the pore. Position of the substituent moiety in the channel varies for different compounds, unlike the fixed orientation in the wild type channel. Here we report a Solid State NMR investigation of the transmembrane domain of S31N M2 (TMD) proton channel bound to the novel inhibitor molecules, while also incorporated into a lipid bilayer environment. Oriented sample solid state NMR experiments of S31N M2 TMD indicate that the channel is sampling two states with helical tilts of 28° and 33° relative to the bilayer normal. The kink in the monomer between two helical fragments, observed in the wild type M2 TMD bound with amantadine, is absent for the S31N M2 TMD bound to all inhibitor molecules tested to date. Stabilizing interactions are further investigated with Rotational Echo Double Resonance Magic Angle Spinning (REDOR MAS) experiments for measuring distances from the inhibitor to the protein.

3338-Pos Board B66

Single Molecule FRET Studies of the NMDA Receptor using Unnatural Amino Acids

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The NMDA receptor is one class of ionotropic glutamate receptors, which are the primary mediators of excitatory neurotransmission in the central nervous system. As such, function and dysfunction of these receptors has been implicated in such physiological and pathological processes as learning and memory, strokes, Alzheimer disease, and Parkinson disease. Studies into the similarly structured AMPA-type glutamate receptor have revealed a relationship between the extent of closure of the agonist-binding domain cleft and activation of the channel, showing a structural mechanism for transduction of signal from binding of ligand to receptor activation. However, neither crystal structures nor more dynamics-based studies using ensemble FRET/LRET show such a correlation when examining the agonist-binding domain of the glycine-binding subunit of the NMDA receptor. Here, we use single molecule FRET to examine more closely the range of conformation states probed by the isolated agonist-binding domain. To perform this, we had to overcome the problem of immutable native cysteines, which resulted in non-specific labeling when using the conventional maleimide-conjugated fluorophores that are regularly used in protein-based FRET studies. Thus, we introduced the unnatural amino acid p-acetylphenylalanine, which contains a unique ketone functional group, at the sites which we wished to test, allowing for specific labeling of the protein. Doing this, we see that while the protein exhibits a peak probability around 33Å when bound both to full agonist glycine and partial agonist ACPC, the protein bound to partial agonist probes a slightly broader range of states than full agonist. From these data, we can infer that while the agonist-binding domain may show similar average extents of cleft closure, irresolvable by crystallography or ensemble LRET, full agonists more narrowly restrict the range of conformational states probed to conformations that allow for channel activation.